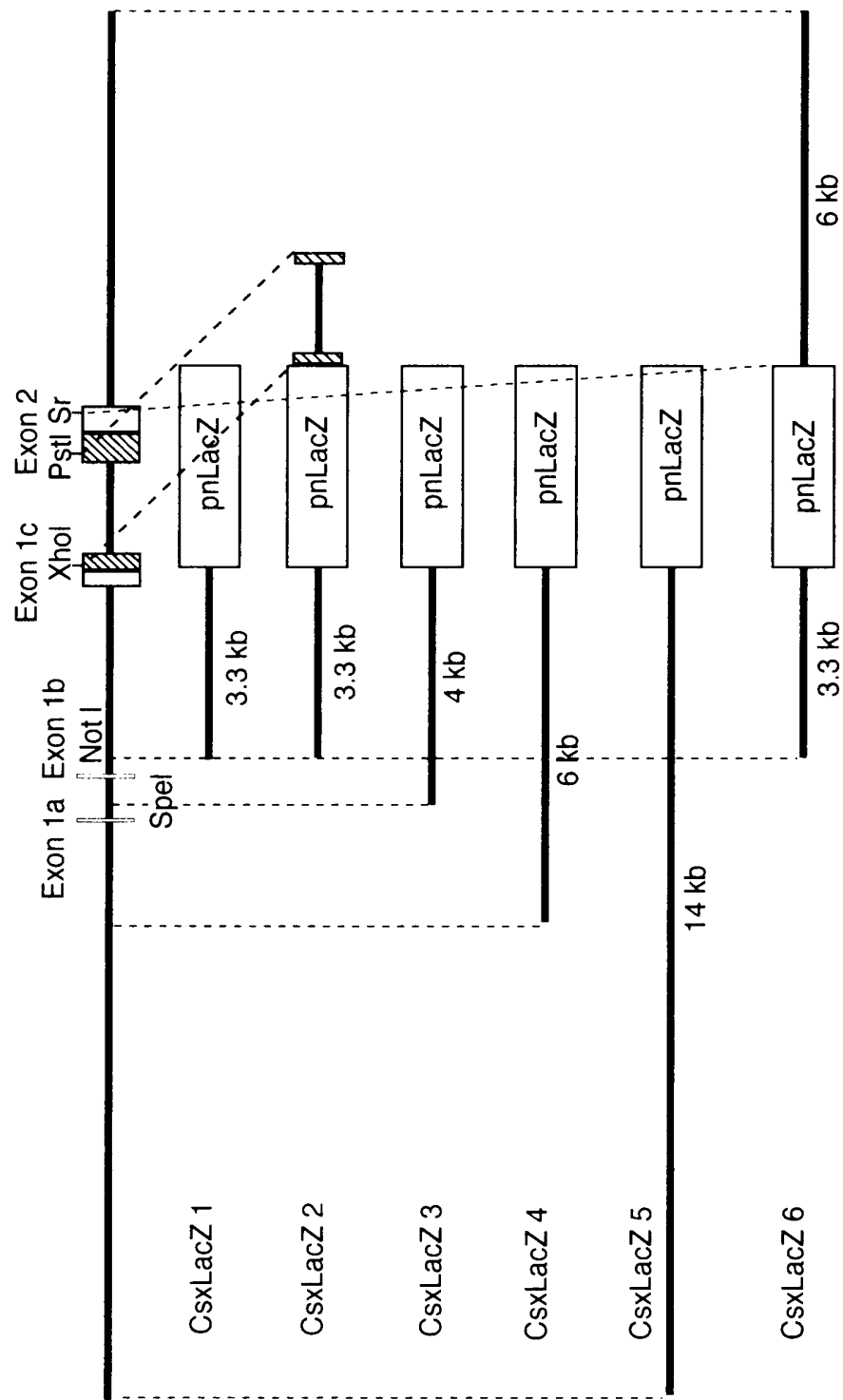
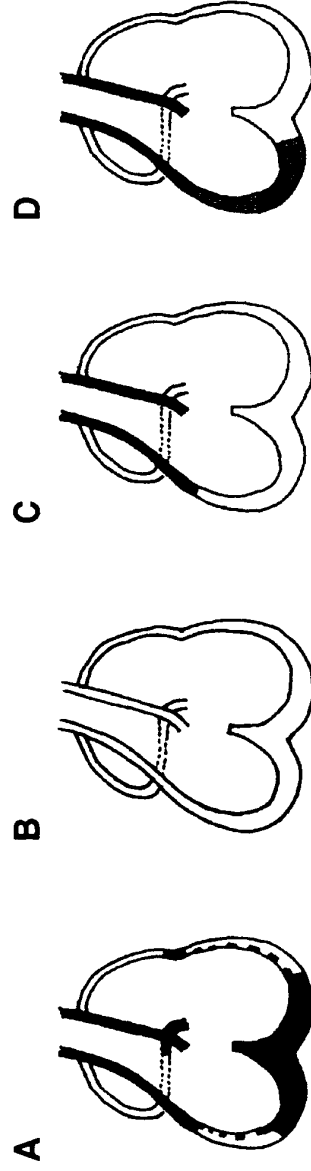


FIG. 1

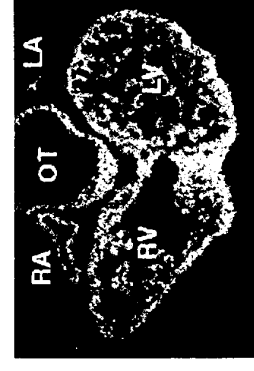
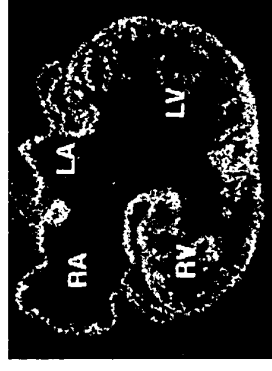
The Genomic Structure of the Mouse *Csx/Nkx2-5*



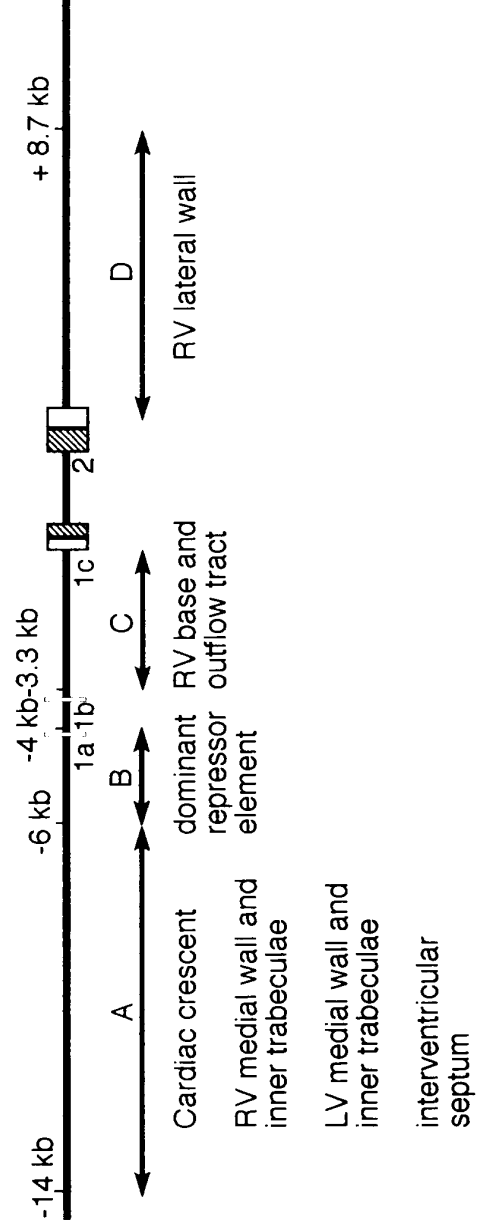
The Locations of the Csx/Nkx2-5 Cardiac Enhancers



Endogenous
Csx/Nkx2.5 at E10.5



Csx/Nkx2.5 locus



The diagram illustrates the genomic organization of the *hsp68* gene and the construction of *hsp68-lacZ* reporter constructs. The top part shows the genomic map with restriction sites (NotI, Scal, XhoI, EcoRI, HindIII, XmnI, PvuII, XbaI, BspI) and exons (Exon 1, Exon 2). The bottom part shows the *hsp68-lacZ* construct with various deletions: 20 kb, 7.5 kb, 3.5 kb, 4 kb, 1 kb, 0.7 kb, and 0.3 kb. The *pmlacZ* construct is also shown.

[illegible]

FIG. 3B

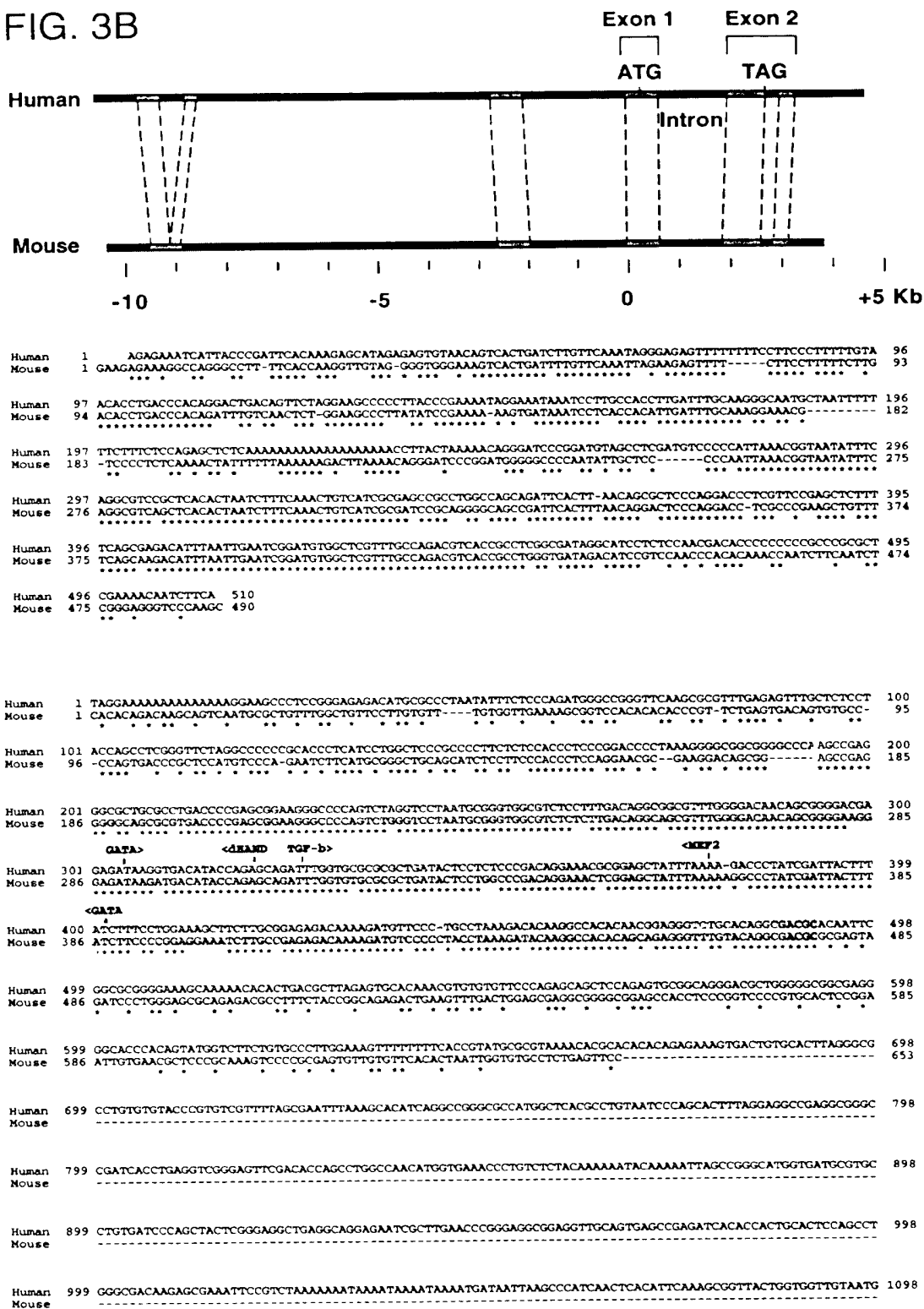


FIG. 3C

**The Genomic DNA Sequence Homology
Between Human and Mouse Csx/Nkx2-5**

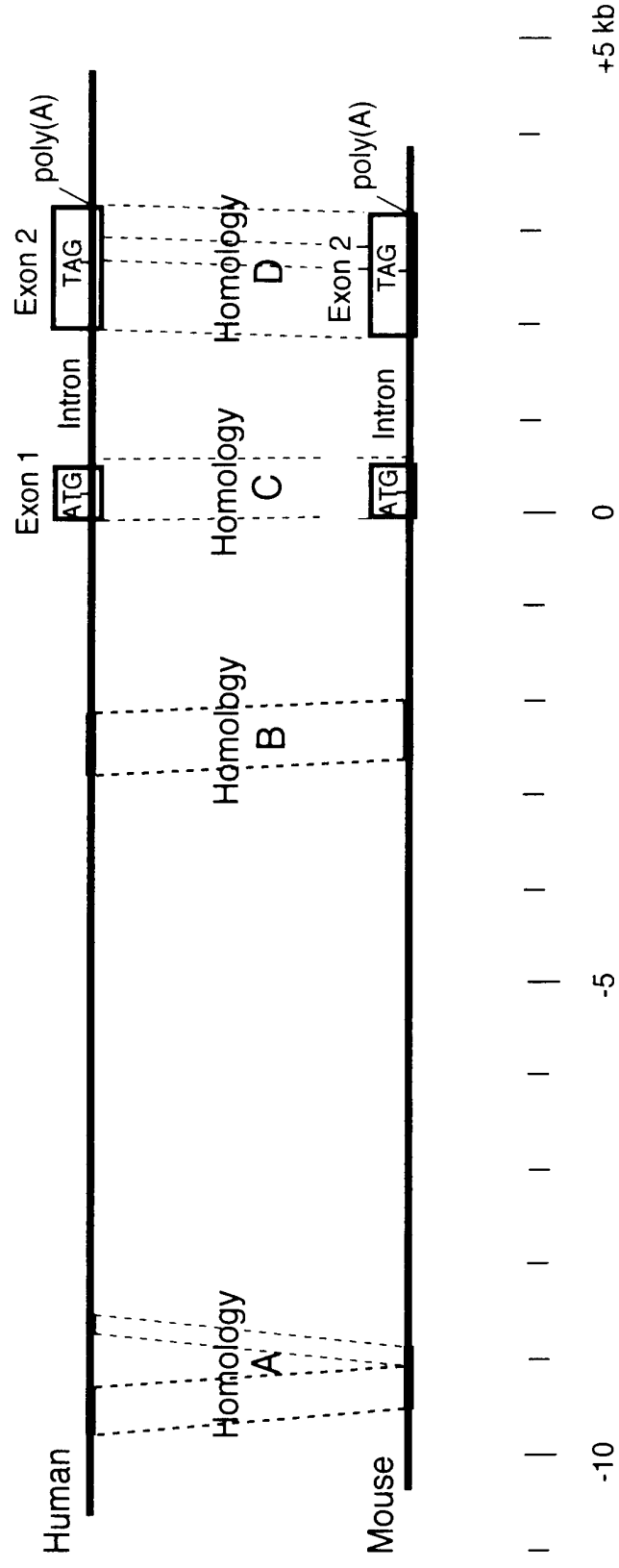


FIG. 4A (1)

CTCGAGCCCAGGAGTTCAAGACCAGCCTGGGAAACATAGGGAGACCCC
TCTCTCTCCACAAAAAATTTAAAAACTAGCCAGGTGTGGTGGCAAACA
CCTGTAGTCCCAGCTACTCAGAAGGCTGAGGTGGGAGGATCACTTGAG
CCTGGAAAGTAGAGGCTACAGTGAGCCGTGATCACACCACTGCACTCC
AGCCTGGGAGACAGAGTGAGACCCTGTCAAATAAAATAACAAACAAAT
AATGATTAAATAACTAAAACTAATTTTATGCTATTTTTCACCTTGTAT
TTTGTAAAGATTTTTTAAATGAAAATTCCCAAATTGCTTTCCAGAAGG
ATTGTTCAAATTATACCCACATTTCACTCATGTTCTCTTCCCTGAACA
GCAGCAATCAGGAAAACTCCCTGGAAGAGGCAGGGCTTAGACTGAGA
TTTTAAAAGGGGGTAGGCCTCAGCTCTCCTTCCAGGTTTACACTGTGC
ATGTTTCCAACTCAAAGAATTTACACTCTTCTGGTTGCATTGCTCTG
TAAAGATCTGACCCACTACTATGTATTAAAAAGGGATGCATGATAATG
AATTCAGCCCTCTCTGTAAAATCCAAAGGGTCCTATTGCAGTTTCCCC
CATTTAATGGGTCATTAAAAATATTCTTGGGAAGGACAAAGCTTTAGTT
AACTATGAGAAAAACAAGCAGAACCAGCCCTGGATTCTGTCTTCAAAG
ATTTTACCATGTTGGCAGGCCTGGTAGTCCAGAGCCCAAGAAAATATC
CCAGCCACAGATACCCTAGATGTAGACTAGCAGTGCTACAACCTCAAG
GTCAGAAGTATGTCACTAGACCAGAGCCAAAAATAGGTGCTATATCAT
TAAGAGAGTAAAAATGCAACCACAGACAGGGTGACATTATTCACAAAT
AAGCATATAACCCACAGGGGACTCCTATCTGAATATGCAAAGAACTCT
CACTAATCAATAAGAAAAAGGCAAAAGATTTAAACAGGCACTTCACAA
AAAAAGTATATTCAAAAAATCAATAAACATTTGAAAAGATCCTCAATT
CACTAGTTATTAGGGAAAGGTGAAATAAAACCACAATGAGACACCCCC
ACGCCCCCACCAGAACGGCTTAAAATCTAAAACATGTAATACCGAATG
TTTGCAAGGATGCGGAGAACTGCCATTTTTGTACACTGCCAGTATGA
GGGTAAATCTGTACAACCAGGTTGGAAAACGCTGAGTAGAATGTACTC
TAGCTGGATTTGTGAATATCATATGATCCAGCAATTCTACTCCTAGAA
ATTTACCCAACAGAAATGTGTAAACATGTTACCCAAAAGACACACGCA
AGACAATTCATAGAGGCACTCACTATTCCTAACAGTCAAAAAGTGGAA
ACTACCCAAATGTCCATCAGCAGAGAATGGCGATAAACAGTAGCATCT
TCACATAATGAAATGTTTCGACAGCAATGAAAAGTAGCTAGCTACAAC
TACAAACAATGTGATTGAACCTCACAAACATATACTAAGTAAAATTAT
CAGACACAAAGAGTGTATATACTGTATTTAGATACATGTGAAGTCTGA
AAACAGGCAAACTATTCTGTTGTTAGAAGTCAGAATAGTTACTGCCC
TGCCGGGAAACAGAACTCAAGAGGGCTTAGTAGCTACTGGTAATGTTT
TGCTTCCCTGAACTGCATGCTAGTGAGGCAGCTGTTATTTTGTGCAGTC
CTGTGTTACACTGGAGTTAAAAGTTCCCCCAAATCAGAAAGTGTTCA
GCAAGTGGAAGCAAGTACACTGCTGGACTTGGCTGGGAACTTAGGGGA
TCCCATAATTTGTACAGGCACAAGCAAAGCCAGCTTTCTTGCCNTAA
GTAGCATCTCCCAGAGTCAGGATCCAGGAATGGTTTGGCAGGCAGGAT
GCAAGGCAGGATTCGGGAGTGGCTGAGAGTTTCCCAGTGCCACCTGG
TCCCACCTCCCCTCTCCCCTTCTAATGAACGGGCAGTACAGCTTCTG
TTAGGAAAAGAGCCTGGGTCCCTAGGCGATGACTGTCACATCTAGGGA
GAGGGCGATGCACTGGGGTCCCTACCTACACCCCCCTTGGCTGTCTCA
CCACTCTGAATTATAAATGCCCGGACTTCCTCATCTCCCACCCACACA

FIG. 4A (2)

TCTTGTTAGAAGAAAAGAAACGAATCTCCCAGGGCTCCTTCTAACAAA
AGTGTTTCATTTCAGAGTAGCCCTGCTTGAGGGCCCCCTGGCCTGGAGGAG
TGGGAGAGGCAGCCCTCCCCCTCCAGGAGAGTCATCTCCAGGGCTACC
CAGGACTGAGTAACTAGGTCAACAGAGTAACCAAAGAGGCAGGAGACA
AGGGCATTCAAGCATTGGGCCAGGAATGGAGGGTGATGTCCAGTTCAT
GTTCTTCTGGTTCCAGCATAGCACACGGTGCAAATGAACCATCATGCA
AGAAAACACAGCTAGTCTCCCTTCCTCCACCAGCAACCTTTGGTACT
GATAATAATCAAATTCATATTTTTTTTTTTTTTTAACTAAGGCTGAG
ATAATGTCAAAGGACCACAGGGAATAGGAAGGCCTAAACCAAGGCCTT
AAAGAATGAGAAGAAGATTCAATCAAAAAAGCCTCCTAAGGGAGGAAG
ATGTTTTTCCCTCCTTTACTTTTCTACAGTAATTTTTATTTTGGATAA
ATAAACCTTGATAAATGAGAACCCACGCTTCCCAAGGCCAGGCTGTG
TTTTGGTGGGTGGTCCTCCGTGAGCAGTTGGAGTAATCCAGAGTGATC
CCGGGCAAGTCGGAAGGGAGCAAGTCTGTGTTGAAGCCAAGAGGTATC
TTTCCCTACAGCTTCTCAAGAGAGGGGATCCCCGTGGGTAAATTGTGAG
GCTGGAAACACCGAGAGGCTGACTCCCATGTTTATAGAGGTCATTGAT
GGGTTTGTGCATGGAAGGCAGGAGGAGACTGAGAGTGCTTTGTTATTG
TTATTTGGTTTATTTTTATTTTTAAAAAACTGGATCAGCCGACTTTGA
ATACAGAAAATGAAAAATGAGGAGATTTGCATAACAGCGCTTGGACGT
CTGAAGGGGGCCAGGGCCTAGCGGCTGGTGGGGCACCTAGAAACACTT
CTGCCTGCAGATCGCGGAGGGTTAGCCACAGGAAGGGGTGCGCTAGGC
TGGCCACAGGGCCTTTGCTGTGACTGAAGGACCAGCCTTGCGGCACC
TTCTTTCCCTCTGCCCTGCACTCCGGCCCCGCGGAGTCAGAGCTGA
CTTGCTGCAGGTGGGGAGAGGACAGAGGCTAGGACGGTGGCGAAACC
TCACCTCGTCGCAGTCCGGAAGGTAAACTTGGACCCGGCAGGCACTTC
CTAAAGTCCAAGCTGCCCTCTCTGAAGAATAAACCTGATTTTCTCCG
GACGCGGACAAAGGAGGATTCGCTCACAACCTAGCCTGTAACAAAGATT
CCCTATTTTCGTGGTTAGGAAAAAAGGAAGCCCTCCGGGA
GAGACATGCGCCCTAATATTTCTCCAGATGGGCCGGGTTCAGCGCG
TTTGAGAGTTTGCTCTCCTACCAGCCTCGGGTTCTAGGCCCCCGCAC
CCTCATCCTGGCTCCCGCCCCCTTCTCTCCACCCTCCCGGACCCCTAAA
GGGGCGGCGGGGCCAAGCCGAGGGCGCTGCGCCTGACCCCGAGCGGA
AGGGCCCCAGTCTAGGTCCTAATGCGGGTGGCGTCTCCTTTGACAGGC
GGCGTTTGGGGACAACAGCGGGGACGAGAGATAAGGTGACATAACCAGA
GCAGATTTGGTGCGCGCTGATACTCCTCTCCCGACAGGAAACGCGG
AGCTATTTAAAGACCCTATCGATTACTTTATCTTTCTTGGAAGCTT
CTTGCGGAGAGACAAAAGATGTTCCCTGCCTAAAGACACAAGGCCACA
CAACGGAGGGTCTGCACAGGCGACGCACAATTCGGCGCGGGGAAAGCA
AAAACACACTGACGCTTAGAGTGACAAACGTGTGTGTTCCCAGAGCA
GCTCCAGAGTGCGGCAGGGACGCTGGGGGCGGCGAGGGGCACCCACAG
TATGGTCTTCTGTGCCCTTGGAAGTTTTTTTTTACCCTATGCGCGTA
AAACACGCACACACAGAGAAAGTGAAGTGTGCACTTAGGGCGCCTGTGT
GTACCCGTGTGTTTTAGCGAATTTAAAGCACATCAGGCCGGGCGCCA
TGGCTCACGCCTGTAATCCCAGCACTTTAGGAGGCCGAGGCGGGCCGA
TCACCTGAGGTGCGGAGTTCGACACCAGCCTGGCCAACATGGTGAAAC

FIG. 4A (3)

CCTGTCTCTACAAAAAATACAAAAATTAGCCGGGCATGGTGATGCGTG
CCTGTGATCCCAGCTACTCGGGAGGCTGAGGCAGGAGAATCGCTTGAA
CCCGGGAGGCGGAGGTTGCAGTGAGCCGAGATCACACCACTGCACTCC
AGCCTGGGCGACAAGAGCGAAATTCCGTCTAAAAAAATAAAATAAAAT
AAAATGATAATTAAGCCCATCAACTCACATTCAAAGCGGTTACTGGTG
GTTGTAATGTATCCATAGACACAGGTCTAAAATGTAAACGCTCCATTG
TGCTCCTTTTAAAGGGCTTGAATGTCTGCAACTGTCATGTGTACACTTA
AAGTATGGGATGTGTCAACACGACCCTTTCTAGCGCGCTCGTTTTCTGTG
TCTGAATCCCCGCATTTCCGCAATTTGCTTGGAGCGCAGAACGCCCTC
CGCGAAAGGCGGCTGCTGATCCCGACTTTTGCTCCGGTATCGCGCAGCT
TGTTGGCCTCCGGGTCCCCCGTGCCATGCCCCGGGAGGCTCTCCACA
GACACCGCTTGCGCCGAATTATACGAGACTGAATGGGTTTTTTTGGTG
TGTGTGTGCAACACAACAATTTGTGAGCTGCTGTTCACAATGCGCTCC
GCCGGGCGGTGGAACTTGGCTGCGGTAACGCACAGCAGGTTGGAGGG
CACGACCCGGAAGGAAGGAAGAGGGCGAGGAGGGAAGGCGGCGACCT
AGGCCCCGCTGGCCAGCCGTTTCCAGCATCAATTCAGCACTGAGCCGGC
CGCAGCAGCACAGGGCTGGGGGCTCCCGGAAGTTCGGCCAGCCGGGGT
TTGGGCCAGAGCCGCGGAGGCTGCCCGGTGGTAGGTGCGACTCTTCAC
CTCTCCGGGGAGCGGCGGCCGACGACCCAACCCACCCGCAAGCGCTGC
CGTCGGCCCCGGCTGGTCCCCCGCGCGGGCACAAAAACAGGCGGCAGTT
CGCCAGCTCTCTTTTCCCAAACCTGAACCGCCAAGCCGAAGGTTCTTC
CAAAGTCGCGGTTCCCCGGGCTTCACACCCGCCGGGCAGGCGCGAACC
AGCCCCAGGACAACCATTTTCTCTTCACTGTATCTGAGTCGTTGTCC
ATCTGACTCGAATGTACCTGATTTTCCAGCTGTGACCTCCAGCGAC
GGGACTCCGAGGAACTGATTCCAGCGTCTCGATTCTCTCCGCCTCTCC
GCCCCGTTTTGGCTGAAGCGGTTTGCAGCCGTCGGGGCAGAAGGGGTGG
GATGTGGCAGCCACCAGCCCCAGCCAGAGAAGAAAAGAGGACGAAAT
TAACGCGAAAGGACACCGGAAGTCTGAAAGCGACTCCCTCGGATCCTC
GGAATCCGAGGCAAACCTAACACTAGTTTGAAAGCGGATCATATCCA
CTAATCCAGGACAAATTCGGGTTGGGAAACATACTCCCCAGAGCCTAA
GAAAACCTGACTTACAACAAAACAAAACCTGACAAGGACAAAATGCAAAG
GAGTTTGTGAAACGTAATTGCTCTCAGAAAATATGTGTATATATATAC
ATCCTATAATATGTTTTAAATTTGCAAAAAAAAAGTCTCTAAGAGGAT
ATATTTTTTAAACCAAGTGGCAGCTTGGGAGGGAGTGGGGATTAGCTGA
GAAGGGGAGAAGGAAGCATTTTTGAGGTGACGTAAATGTTTTTGTATC
TTGATTATGGTGGCTGTTATGGGGGTGCACATCCAAGTGTCAAGACTC
ATCGAACTGTACACTTTTGTCTAGGTACATTAGACCTCAATAAAGTG
GATTTTAAACCTAAATAAGCCAGGTAACAGCTTTGCCTGGGTGGCTGG
GGGAGAGGCTTGGGACACTTTACATTGATCTCCCTCTTAGGCATGTTT
GTTTTGGTTTGGTTTTGTTCTTATGATGTATTATTTATTCAAAAATAT
ATCATTAGCAGAGTGAATGTAAATGTAAAACCATTGTTAAGGAAA
CCAACAAAAGCGGGAACAAGAGACACTGGTGCATCCTGTTAGAGGGAT
AAGAATAAGCACTCGCTGTCCAAGCTCATAAAATATTTTGGGAATGAA
TGTCGTTCCGCTTTGTTTTTTTTGGTTTTTTTTTGCTCATGTGTTAACAT
CAACGAGAAATGAGGACCCAAAACCTTATCCAGTGGTTACGTGTGGTGT

FIG. 4A (4)

GTGTGGCTGTCATCTCCTTGGGACTGGCTACTGAAGGCCACAGGCGTG
GGAGGACCAAATGCTCCCTGGATGTTGAGTCCCAGCCGGTAAGCAGCA
CACAGTCCCGCTTGCAGCAAAGATGTGGTGGCCGGCTGCGCTGTGGGG
GAAGGCCAGGCCCCGACAGGAACCTCAGATCTCACCGGCGGATGAGAG
TGGTGGCCCCCTGCAGCTGGAGTCCCTGCTGGCCTGAGAGCTCCAGCTG
TGCCACCGTTGGGCAGACCCACACTTCAGGGAGCTGCCAGGATCAGT
GGCTACAAGAGTCCCCACCGTGTTTGGAGAACTAGGTATGAAATATT
TCCATTTACACCCCTACCCGGCCCCAGACAGGAAAGTCACTTCAACC
TTGTTAGGTCAGATTCCAGATCTGGTTCAGATGCAGGGCTATTTCAGA
GAGATTTTTAGAGGCTGACTCTCAGGAGAGGGAAGGACAGTGGGCTGA
AGGCCAGGGGTCAGGAAATCTAGGAACTGCTAAACTCCTCTGCTGGCC
TGCGGGGAGCGCCCCGGGTGGGGCTACCAAGGCCACAAGCCAGTTCCAT
CTTCCCACCTTTGCCACCTTCTCACAGGGACCAGGCTCTGCATCCTCAG
TGACCACAAGACTTGGGCCTGCCCTCTAGTTTGTCTATACCTGCCCCC
TCCCTTGACTCATACTGTCCAAGACCCCAAGACCAAACCACAAGTCAG
GAGAGATCTTGAGGGCAGCCAGTGCCACCAGGGTCCTGTTCCCAGGTA
CTACTAGACAAAGGCCACCCTTCCTCCCCTCTCTCTAGGGCTCCGCTG
ACCACCCTGCACAGTCTTCCTACACCAAGGGCTCCGGTGCCACCCCTT
CACAGAGAGTTCACTGCACCGCTGCTTCGGCTGCCTGTCTCAAACCAT
ACACACACCTTTGATTCTTAAACTCCAAGATTAGGATGGGCCCCAGAA
ATCTGCATTTTTAATATGTACCTCAGAGGATTCTGGCCTAGATATTTT
TACAGCCCCAAAAGTAACAAGGAACCTGTTCCAAAAAGTGATTACGG
AAACTGTCATGTTTATTCTTGACTTGCCCCCAATTATTCTTCCCCTG
AAGTTTTTCATCACCAAAAAACCCACATGTGAACCATATGTGTACATA
TGCCCATATTTAAAATACAAATTCTGCACCTGGTTTGCTATTTAAAGT
ATCTCAAAACATATCCATAAGAATACATATGAATGGAACATAATTCTTT
CTCATGGGATATGGGATCTGTTCTATGGACAACATAATTTTAAACCAG
TCCTAGTATATATACACTGGTTTTTTTACATGTTGATCTTAAAAAATAA
AAACGGNTGAAA (SEQ ID NO.: 4)

FIG. 4B (1)

CAATTTCTATTNAGTTCTATTAAAAGGGATTTTTTTTNAACTCACTGGNAACCAGGAGGA
CTGNAAAGAAAAGTGAAATGGCTCTGGGACTTTCCTCTAAGGAGACCAGCATGGGTCCG
CCAATTTTTATTGTCACGTATTTGTCCGTTTTTGCCCCATCTCCTCTCTCCTGAAACAC
CAAGACCTTTTTTGAAGCCAAGAGAAATCATTACCCGATTACAAAGAGCATAGAGAGTG
TAACAGTCACTGATCTTGTTCAAATAGGGAGAGTTTTTTTTTCCCTTCCCTTTTTGTAAACAC
CTGACCCACAGGACTGACAGTTCTAGGAAGCCCCCTTACCCGAAAATAGGAAATAAATCC
TTGCCACCTTGATTTGCAAGGGCAATGCTAATTTTTTTTCTTTCTCCAGAGCTCTCAAAAA
AAAAAAAAAAAAAACCTTACTAAAAACAGGGATCCCGGATGTAGCCTCGATGTCCCCCAT
TAAACGGTAATATTTTCAAGCGTCCGCTCACACTAATCTTTCAAACGTGCATCGCGAGCCG
CCTGGCCAGCAGATTCACTTAACAGCGCTCCAGGACCCTCGTTCGAGCTCTTTTCAGC
GAGACATTTAATTGAATCGGATGTGGCTCGTTTGCCAGACGTCACCGCCTCGGCGATAGG
CATCCTCTCCAACGACACCCCCCCCCCGCCGCGCTCGAAAACAATCTTCAAAGGCAAGG
GGGCCCCCAAGTAGGTTAATTTACAACCATAACGGTAACGTGGCCAAAAGNCAGGCGAG
GAAGGGCCGCAAGGCCGCTGACATGCAAGCTCCGTCCAAGAAGAATTTGGGTGGAGGTG
AAGAGGTGGGGGGACGAGGTTTTCNTGGGCCCTTGAACGCCCCACATTTAAAAAAGGCATCC
TCCACAGACTAGACTAACAAATCCAGACCCCCAGTAGTCCCTGGCTCAGAACTCGAGGC
GTGATTTGCGCGTGGCAGCCAGGCCTGTTACTGACGGCTGGCGCCTAGAAGCCGGGGTC
AGGGCGTTGCGCGCCTCCTGGGCTGCCCTGCGGGGCTCACCTCTCTCCCAGCATGGAGG
CCCCAGGTCTGGGAGTGTGGCTTTGATGAGGGACAGGAAAAGTCCCAACATCAGGCCAA
TGCTTGACTTCACTTGCGTCGGCGTCTCAGACGGCACACTGTGCGGTGTTGAGCACCCAAG
ATGTACGTTCTGGACAGACACTATTTTGTCCCCATACATGGAGCGTTTCCCTCCGCACCTT
GGGCGCGCCTGCGGGAGCTGTGTCTTTAGGTAGTTTTTGGCCCTGCGCCGCCTTTATTCT
ACTCCAAGCGCTCTTTGCCAAACCCGCACTCCGCAAAGAGCCAAGCCCTCCACATCCCCA
TTCTCAGCAAGTCCACGCGTCCCGCCCAGCTTCCCGCCCGCGGTTCCCTGTACCAGCTAG
GGCCGTGAGAAGCCAACGCTTTTCCACTGACAAATCCTGTATCCCCAGCTCTAGAAGGC
GTCCTTAACCTGGGCCCCGCTCTGCCTGCCCGGACTCCTGAATTGTAAGCAAAATAAACT
CCTCTCTGCAGTGTTCTGGGGAATGGAGAAGACCCCAAGCTTTCATCAGACCCCTCCCAAG
GAGTGCGGGGACCCAGAGAAATGAGGCCACCCGGGCAGGATCTGGCCATGTAGCTGGCGC
TCCTGAAACTCTGGCAGATTTGTCTGACTTCTGTGCCCTACTCTACTGACCCTGGGCTAA
AAATGATCATGATCACCCCACTTGCCCTGCCCTTCCCCACGCGCCTGACCGAGCCGCAG
GGGTGCCCCACTGGAAGTCCGGCCCAGAGGCCTCAGAGAAATCCTGGCCTAGCTGGGCTC
AGAGGAGCCCCGCCTCCCTGAGAGCTAAACCTGGGCTAGGACCCTGAAACCTCGAGGTTG
GCAGAAGCCTGAGGGCCTTGCTGCCAGGCAGGGAGGGCACGGGAAGGAGGGAGGTGGGAT
CGATGGCCTCCAAACAGGGGAAACAAGGTGGCTGGTAGCTGGGGCACTCCACAAGACAGG
TGTNTCCTGGGAAGCTGAGCTTACCAGCTGGGATTCTGATTTATTTATTATTAAGGGG
AGAGGCATTTCCCTGGGAGGGTACTGGCAGTGAATGATGCCCCCTGGAGTTGTGCTGTG
CATAACACTACTGTAGGAGGCAGCAACTCCTACCCACCTGGCCATCACTCACCTTGCCC
TTACTTTCTGTTGATTCGCCCAGAAGCACCCAGAGCCTGCGGCATGATTGACCCTGTAGGC
CAAGCCAAACCAAACCCCGAATTGTCCAGAATTTTCGCCCTGGTGTATCCCCAAAGCCC
AGCCCTGTCTTTNAGGGTTTTTTTTTCTATTGAGATTTTCCCTCATCCCACCACCTTTAGT
AATAAAGCCTTCCTCAAACATAATTTCTCCTCCACACCGCTTCCACCCCATCCTTTTTTTTT
CCCATGCTGGTTTGGGTGCTGAGGAATATTTTTTCAAACCCACACCCATCCAGCCCTGCC
CAGAGGCCTGACTTTGCATGCCTCTGGTAGGNTTTTCAGGGTTACATTAGGGAGCAAAAG
CAGGGTGCAGGGGCAAAAGGGGACCCTTCAAATGGGTGCGTGGCCCCCTTTAAAAAAGCTG
GGCAGGGNTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGGCGTATGACTATA

FIG. 4B (2)

TTAGGTGACACGAAACTGCTCATCGCTCCTGTTCATCGAGGCCCTGGCCCAATGGCAGGC
TGAGTCCCCCTCCTCTGGCCTGGTCCCGCCTCTCCTGCCCCCTTGCTGCTCAGCGCTACCTG
CTGCCCCGACACATCCAGAGCTGGCCGACGGGTGCGCGGGCGGGCGGCACCATGCAG
GGAAGCTGCCAGGGGCCGTGGGCAGCGCCGCTTTCTGCCGCCACCTGGCGCTGTGAGAC
TGGCGCTGCCACCATGTTCCCCAGCCCTGCTCTACGCCCACGCCCTTCTCAGTCAAAGA
CATCCTAAACCTGGAACAGCAGCAGCGCAGCCTGGCTGCCGCCGGAGAGCTCTCTGCCCCG
CCTGGAGGCGACCCTGGCGCCCTCCTCCTGCATGCTGGCCGCCTTCAAGCCAGAGGCCTA
CGCTGGGCCCCGAGGCGGCTGCGCCGGGCCTCCAGAGCTGCGCGCAGAGCTGGGCCGCGC
GCCTTCACCGGCCAAGTGTGCGTCTGCCTTTCCCGCCGCCCGCCCTTCTATCCACGTGC
CTACAGCGACCCCGACCCAGCCAAGGACCCTAGAGCCGAAAAGAAAGGTGAGGAGGAAAC
ACAGGCCCCCTTCTCCCCCTCCTGGGTGCTTTCGTCCCCAAGAACTCAGGGCCAGGAGG
AGGACACACGCGCCCTTGGGCCGAGGGCTGGGCTGCGGCGGGGGGTTCAGAATGTAAGAT
GCCTGGTGTTGTCGCCAGGCTCCCGCGCCCCGCGTCCAATCGGAGGTTTCAAGGAAATGC
CGGATTGAAAGGATCCGAAAGCAAGAGACCAAAAACTTTTCCCCCGGCCCTAACAAACC
CCCGGCGGTTTCCGCTCTGCTCCTGGTTCTGGTAGAATTTTAAAAATCGGTTTATGTTTA
AACAAAACAAAAAACAGCCAAAACCCCCGTTTTTTTACCCCCCCTTGGATTTTCAAACC
CTTTTTTAAATTTTTTGAAAAAAACCCCCAAACAAAATTAATTTTTTCCCCCAAAAAAT
TTTTTTTTTTTAAACAAAAGGGGGGGTGGAAAATTTTTTTTTTCCCCCCCCCAAAAGGGGTT
TTTGTTTTTTTTTTT - - - - - TTTNTTTGGCAAAAATGAATTNTGGANCNAGGCCTTAT
TTNAAATGGATATTGGGNCCNCAGGATTTTGATTTTCAATTTATTTTTTTAAGCAAACCTNC
CGGGCCGGCAAGGGGAAAGGTTCCCTCGTGGAAAAGTAGGAAATGCTGCGCTACCGCGGG
CACAAAGGNAGTGGACGAGATGAGTGCGGGATCATCCCGCAGGCCATCCAGGATCGGGGA
GGGAGGCCCGCCCCGCTGCAGAAAGGGGCTTCTGGGAGACCCCCAGCCCAAGGCAGGAG
CCCGGGCGATTCCCGGGAGGCCGCGAGGCGCTGGGCGAAGCGCTGGGCGAAGGGCCGCTGC
CAGCCGGGAGAGAATTCATAGGTTTGTGAGGAGCAGAGGCCTGGGAACAAATTCGGGCG
GGCACGGCGGCTAGAACTGATCGCTACCAATTCGAGGAAGCCAGCAAGGCAGGTTCCGAG
GCCGCTGCCACCCGCGAGCTTCTTGACACTGCGCAAACCTGCTGCGGCCAGGCTGGA
GCCTCCGATACCAAACCAACACTCCCTGGCCTTCTGTTTCTTGATTCTTAATTTTGAG
ATAAGACCGTCCCTAGCAGTGAGGCCTCGGCCTCTGTTCAATTTAACTTCTCAAACCAAAC
TAGCCCTAATTCAGTTCACCCAGAGCATCACCTGGTTTTATTTTTTATTTTTTTATTTTTT
TTATTTATTTTTTTTTTTTTTTTGCAGCCTGAAATTTTAAGTCACCGTTTGTCTCCCTCACC
AGGGTGTGAACTGCCCCGAGGGCAGAGACCTCCCGTTTTGTTTTCCAGCGCCTTGAGCCA
GCTTGACTTTTTTACAAATGCTGAGTGAGACGTGTCGGTGGCTCCAGTGCACTTGGCAGA
GTGAGCCGCAGCCAGCTGGGCGCTCCAGGCAGGACACAGTGGCCTCCACGAGGATCCCTT
ACCATTACTGTGCGGCCGCGCTCCGTAGGTCAAGCCGCTCTTACCAAGCGTCTTTCTGCC
TTTCTGTTCCCCCTCAGAGCTGTGCGCGCTGCAGAAGGCGGTGGAGCTGGAGAAGACAGA
GGCGGACAACGCGGAGCGGCCCGGGCGCGACGGCGGAGGAAGCCGCGCGTGCTCTTCTC
GCAGGCGCAGGTCTATGAGCTGGAGCGGCGCTTCAAGCAGCAGCGGTACCTGTGCGCCCC
CGAACGCGACCAGCTGGCCAGCGTGCTGAAACTCACGTCCACGCAGGTCAAGATCTGGTT
CCAGAACCGGCGCTACAAGTGCAAGCGGCAGCGGCAGGACCAGACTCTGGAGCTGGTGGG
GCTGCCCCCGCCGCCGCCGCGCCTGCCCGCAGGATCGCGGTGCCAGTGCTGGTGCGCGA
TGGCAAGCCATGCCTAGGGGACTCGGCGCCCTACGCGCCTGCCTACGGCGTGGGCCTCAA
TCCCTACGGTTATAACGCCTACCCCGCCTATCCGGGTTACGGCGGCGCGGCCTGCAGCCC
TGGCTACAGCTGCACTGCCGCTTACCCCGCCGGGCCTTCCCCAGCGCAGCCGGCCACTGC
CGCCGCCAACAACTTCGTGAACCTTCGGCGTCGGGGACTTGAATGCGGTTTCAAGAGCCC

FIG. 4B (3)

CGGGATTCCGCAGAGCAACTCGGGAGTGTCCACGCTGCATGGTATCCGAGCCTGGTAGGG
AAGGGACCCGCGTGGCGCGACCCCTGACCGATCCCACCTCAACAGCTCCCTGACTCTCGTG
GGGAGAAGGGGCTCCCAACATGACCCTGAGTCCCCTGGATTTTGCATTCACTCCTGCGGA
GACCTAGGAACTTTTTCTGTCCCACGCGCGTTTGTTCCTTGCGCACGGGAGAGTTTGTGGC
GGCGATTATGCAGCGTGCAATGAGTGATCCTGCAGCCTGGTGTCTTAGCTGTCCCCCAG
GAGTGCCCTCCGAGAGTCCATGGGCACCCCCGGTTGGAAC TGGGACTGAGCTCGGGCAGC
CAGGGCCTGAGATCTGGCCGCCCATTCGCGAGCCAGGGCCGGGCGCCCGGGCCTTTGCT
ATCTCGCCGTCGCCCCGCCACGCACCCACCCGTATTTATGTTTTTACCTATTGCTGTAAG
AAATGACGATCCCCTTCCCATTAAGAGAGTGC GTTGACCCCGCACGTGTGCTTCTTTCA
GCTTGCGGCGCTTCAGAAGCAGGAGAGAGGTGGCCGCCCGGGA CTGGTCTCAGATCTCAG
GCACAGGCATTCCCTGAGCAAATTGATAACATTGATACTAATAAAACCTAACCCCTTGCTG
GAACCATACTGGTTCCGTGTCGGGCAC TTTCTGAGATTGTCTCATATAATCCTCAATAAT
CCAAAAAAAAAAAAATCCTAAAGTTTAGAAGCTGAGGCCCGGAGAGGTTTAAATGACTTAC
CTGCGAGCAAATAGCCAGTACTAGTCGA ACTCTGGTTAAATTCAGGATGCCTCACTTCAG
AGACCGCCTTCCCTGTGCTCCCAAGCTCCCCTCCTTGAATCCTAATGTGTGCCAGGCACG
GTTCCAGGCACTGGGCATTAAATGGACAAGCAAAAGAACCTGGGCCCTCTGTAGCTGGAG
AGCACCGTGATCATCCCACTTAAAGAACTCCTTAACCTGTTTCCAAGATGGNAAAAGCC
AAGAANCCAAAGCCCTTGGGNAAGCGTTCTCAAGGGTCCTCANATGCCCCAAATGCCACG
TCGGGGGCTCAACANCTNGCCCGTTGGAACTGAATGCCNANGGTGGGCCCCAAANAAGGN
TCCTGCGGGATGGNGCTCAACTCCAAGCTGTGGTGAAGGCCCATAAAATTCAAATGGGCC
AAGGGGAGCCCCCTAAAGCCCTAAACCTTCNGGGGGTCCNTTCCCTAAGGGCATTTAANT
TTACCAAAGTTTGGNCAAANAATGTTTCCAATGGNCCNGATTTTATNGANGGGNAAAAC
TGGNGGGCAACCGAAATCCAGTTTAAACCCGGGTGTGTTT (SEQ ID NO.: 5)

FIG. 5A

AGGCCCCCG CACCCTCATC CTGGCTCCCG CCCCTTCTCT CCACCCTCCC
GGACCCCTAA AGGGGCGGCG GGGCCCAAGC CGAGGGCGCT GCGCCTGACC
CCGAGCGGAA GGGCCCCAGT CTAGGTCCTA ATGCGGGTGG CGTCTCCTTT
GACAGGCGGC GTTTGGGGAC AACAGCGGGG ACGAGAGATA AGGTGACATA
CCAGAGCAGA TTTGGTGCGC GCGCTGATAC TCCTCTCCCG ACAGGAAACG
CGGAGCTATT TAAAAGACCC TATCGATTAC TTTATCTTTC CTGGAAAGCT
TCTTGCGGAG AGACAAAAGA TGTTCCCTGC CTAAAGACAC AAGGCCACAC
AACGGAGGGT CTGCACAGGC GACGC (SEQ ID NO.: 1)

TGCTCCTTT TAAGGGCTTG AATGTCTGCA ACTGTCATGT GTACACTTAA
AG (SEQ ID NO.: 2)

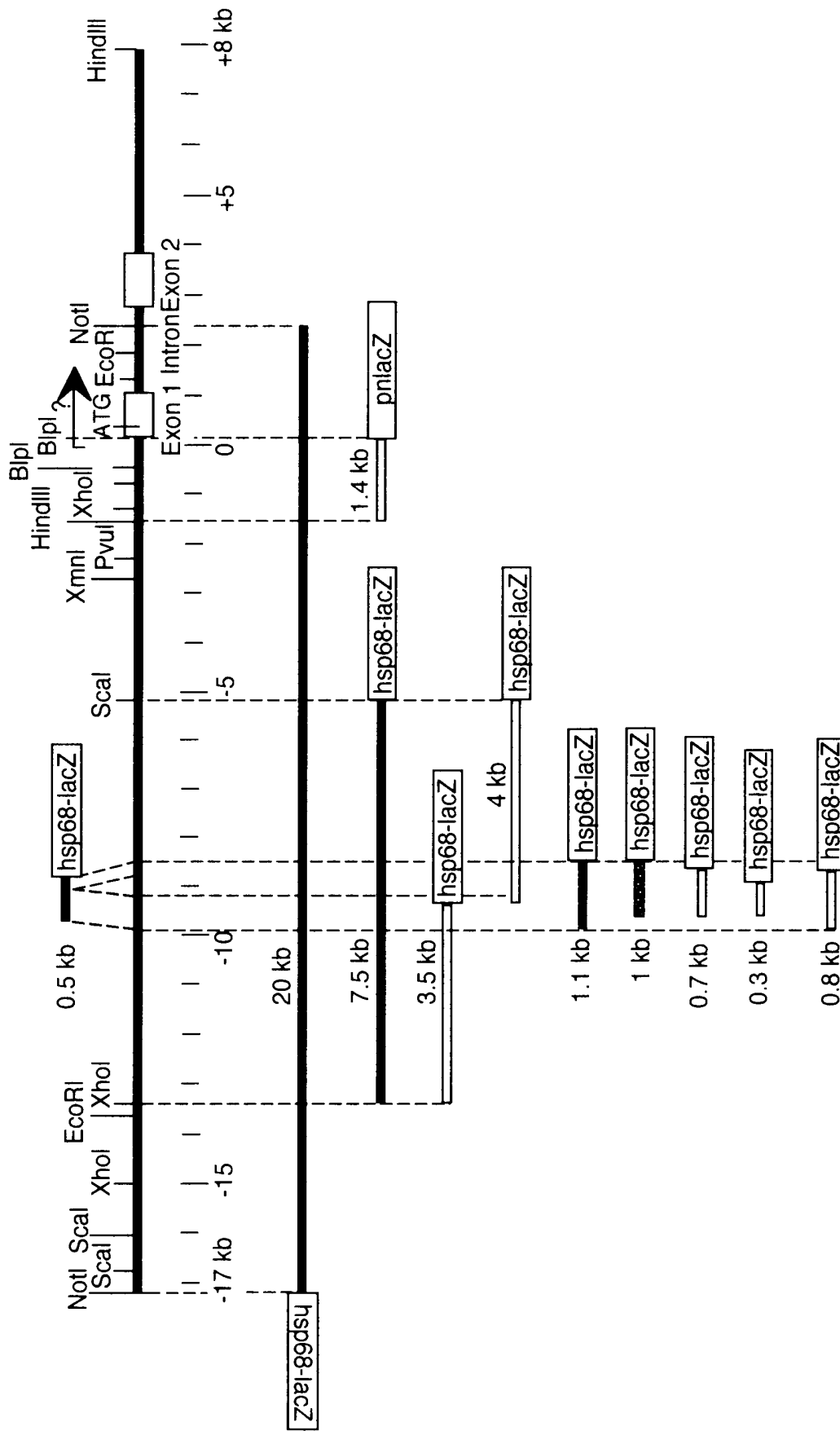
FIG. 5B

AGGCCCCCG CACCCTCATC CTGGCTCCCG CCCCTTCTCT CCACCCTCCC
GGACCCCTAA AGGGGCGGCG GGGCCCAAGC CGAGGGCGCT GCGCCTGACC
CCGAGCGGAA GGGCCCCAGT CTAGGTCCTA ATGCGGGTGG CGTCTCCTTT
GACAGGCGGC GTTTGGGGAC AACAGCGGGG ACGAGAGATA AGGTGACATA
CCAGAGCAGA TTTGGTGCGC GCGCTGATAC TCCTCTCCCG ACAGGAAACG
CGGAGCTATT TAAAAGACCC TATCGATTAC TTTATCTTTC CTGGAAAGCT
TCTTGCGGAG AGACAAAAGA TGTTCCTGCG CTAAAGACAC AAGGCCACAC
AACGGAGGGT CTGCACAGGC GACGCACAAT TCGGCGCGGG GAAAGCAAAA
ACACACTGAC GCTTAGAGTG CACAAACGTG TGTGTTCCCA GAGCAGCTCC
AGAGTGCGGC AGGGACGCTG GGGGCGGCGA GGGGCACCCA CAGTATGGTC
TTCTGTGCCC TTGGAAAGTT TTTTTTCACC GTATGCGCGT AAAACACGCA
CACACAGAGA AAGTGA CTGT GCACTTAGGG CGCCTGTGTG TACCCGTGTC
GTTTTAGCGA ATTTAAAGCA CATCAGGCCG GCGCCATGG CTCACGCCTG
TAATCCCAGC ACTTTAGGAG GCCGAGGCGG GCGGATCACC TGAGGTCGGG
AGTTTCGACAC CAGCCTGGCC AACATGGTGA AACCCTGTCT CTACAAAAAA
TACAAAAATT AGCCGGGCAT GGTGATGCGT GCCTGTGATC CCAGCTACTC
GGGAGGCTGA GGCAGGAGAA TCGCTTGAAC CCGGGAGGCG GAGGTTGCAG
TGAGCCGAGA TCACACCACT GCACTCCAGC CTGGGCGACA AGAGCGAAAT
TCCGTCTAAA AAAATAAAAT AAAATAAAAT GATAATTAAG CCCATCAACT
CACATTCAA GCGGTTACTG GTGGTTGTAA TGTATCCATA GACACAGGTC
TAAAATGTAA ACGCTCCATT GTGCTCCTTT TAAGGGCTTG AATGTCTGCA
ACTGTCATGT GTACACTTAA AG (SEQ ID NO.: 3)

FIG. 5C

AGAGAAATCA TTACCCGATT CACAAAGAGC ATAGAGAGTG TAACAGTCAC
 TGATCTTGTT CAAATAGGGA GAGTTTTTTT TCCTTCCCTT TTTGTAACAC
 CTGACCCACA GGA CTGACAG TTCTAGGAAG CCCCCTTACC CGAAAATAGG
 AAATAAATCC TTGCCACCTT GATT TGCAAG GGCAATGCTA ATTTTTTTCT
 TTCTCCAGAG CTCTCAAAA AAAAAAAAAA AAAACCTTAC TAAAAACAGG
 GATCCCGGAT GTAGCCTCGA TGTCCCCCAT TAAACGGTAA TATTT CAGGC
 GTCCGCTCAC ACTAATCTTT CAAACTGTCA TCGCGAGCCG CCTGGCCAGC
 AGATTCACTT AACAGCGCTC CCAGGACCCT CGTTCCGAGC TCTTTTCAGC
 GAGACATTTA ATTGAATCGG ATGTGGCTCG TTTGCCAGAC GTCACCGCCT
 CGGCGATAGG CATCCTCTCC AACGACAC (SEQ ID NO.: 6)

FIG. 6 Transgenic Constructs of the Human Csx/Nkx2-5 Enhancer



Seq ID No: 5

Seq ID No: 4

FIG. 7

Transgenic Analysis of the Human Csx Enhancer Sequence

<u>Constructs</u>	<u># of Transgenes</u>	<u>Enhancer positives</u> (Cardiac : Ectopic) ¹
20 kb	8	4 : 0
7.5 kb	8	6 : 1
promoter-proximal 4 kb	7	0 : 1
promoter-distal 3.5 kb	6	0 : 0
1.1 kb	8	3 : 1
1.0 kb	10	1 : 2
0.7 kb	8	0 : 3
0.3 kb	11	0 : 6
0.8 kb	6	0 : 1
0.5 kb	2	2 : 0

1. Each embryo was classified into either 'cardiac' or 'ectopic' judged upon the extent of similar to the endogenous Csx expression pattern.

ALL INFORMATION CONTAINED

Embryo 4
E10.5Embryo 3
E10.5Embryo
E10.5Embryo 1
E10.5

20 kb

Line 1
E12.5

Embryo 8	Embryo 9
E9.5	E10.5

Embryo 7
E10.5Embryo 6
E11.5Embryo 5
E10.5

7.5 kb

Embryo 9
E9.5

5.6.1

1.1 kb

RA; Right Atrium LA; Left Atrium RV; Right Ventricle LV; Left Ventricle
Ph; Pharyngeal Arch St; Stomach OT; Outflow Tract

Cardiac Expression of the 7.5 kb hCsx Enhancer-hsp68-lacZ Construct



4 Weeks

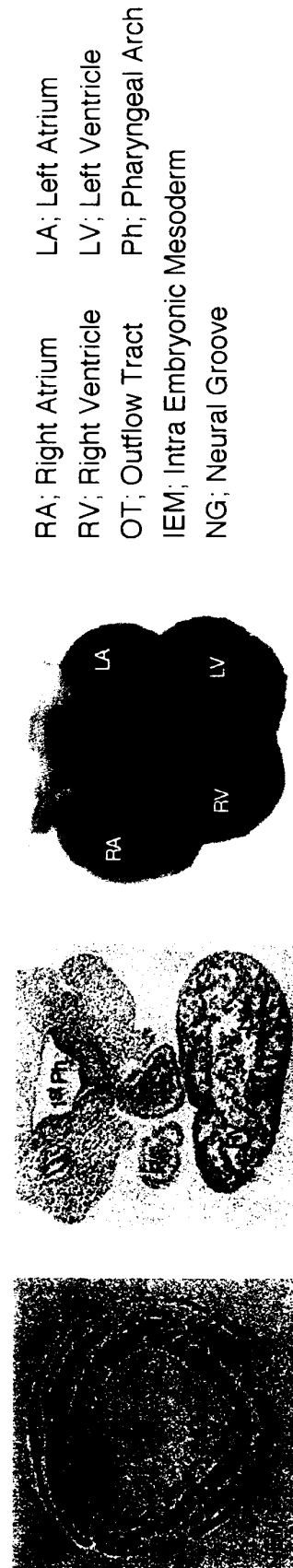
3 Days

E12.5

E10.5

5.63

E7.5



E7.5

E10.5

E12.5

RA; Right Atrium
RV; Right Ventricle
OT; Outflow Tract
IEM; Intra Embryonic Mesoderm
NG; Neural Groove
LA; Left Atrium
LV; Left Ventricle
Ph; Pharyngeal Arch

FIG. 10

Facilitated isolation of cardiac myocytes

